APPENDICES

FOR

UNITED STATES LETTERS PATENT

TITLE: **OVERCOMING DAPA AMINOTRANSFERASE BOTTLENECKS IN BIOTIN VITAMERS BIOSYNTHESIS**

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Appendix I. Medium composition for biotin and vitamers production in bench scale fermentors.

Medium Component	Concentration	
	Batch	Feed
Glucose	15.0 g/liter	750 g/liter
Veal Infusion Broth[1]	25.0 g/liter	---
[redacted]t Extract[1]	5.0 g/liter	---
Sodium Glutamate	5.0 g/liter	---
KH_2PO_4	7.5 g/liter	13.7 g/liter
$MgCl_2 \cdot 6H_2O$	1.0 g/liter	1.5 g/liter
$(NH_4)_2SO_4$	2.0 g/liter	---
MAZU DF-37C	2.5 g/liter	---
$CaCl_2 \cdot 2H_2O$	1.0 g/liter	---
$CuSO_4 \cdot 5H_2O$	0.4 mg/liter	4.0 mg/liter
$ZnSO_4 \cdot 7H_2O$	0.5 mg/liter	5.0 mg/liter
$MnSO_4 \cdot H_2O$	25.0 mg/liter	35.0 mg/liter
[redacted] $6H_2O$	1.0 mg/liter	10.0 mg/liter
Sodium Molybdate-$2H_2O$	0.2 mg/liter	2.0 mg/liter
$FeSO_4 \cdot 7H_2O$	50.0 mg/liter	100.0 mg/liter
Sodium Citrate-$2H_2O$	50.0 mg/liter	100.0 mg/liter

[1] In Amberex Medium the Veal Infusion Broth and Yeast Extract are replaced with 10 g/l Amberex 695.

Appendix II. Protocol of avidin-HABA [2-(4-hydroxyphenylazo) benzoic acid] displacement assay for biotin and dethiobiotin.

Reagents and Solutions:

Buffer:	0.1 M $NaPO_4$, pH 7.0.
Avidin:	From Sigma (Cat # A-9275). Dissolved at 5 mg/ml in Buffer.
HABA:	From Aldrich (Cat # 14,803-2). Dissolved at 0.375 M in water + 1 eq. NaOH.

Prepare Mix:

	20 samples		50 samples	
Avidin	1	ml	2.5	ml
HABA	0.08	ml	0.2	ml
Buffer	38.9	ml	97.3	ml

Assay:

Zero spectrophotometer;

Add 2 ml of Buffer to disposable 5 ml cuvette; record OD_{500}.

To read sample:

Place disposable 5 ml cuvette in spectrophotometer.
Add 2 ml of Mix; stir; record OD_{500}.
Add sample in 0.1 ml volume; stir; record OD_{500}.

Standards:

Use 0.1 ml DTB at 2 mg/ml to 14 mg/ml as samples.
Use 0.1 ml Buffer as "zero" point.

Calculations:

Calculate ΔOD_{500} minus ΔOD_{500}.
Plot standards and use curve to determine HABA vitamers from samples.

Notes:
1. Useful range is 2 to 14 mg/l of biotin + dethiobiotin.
2. Add mix to cuvette, read OD500, and then add sample and mix without removing cuvette from the spectrophotometer.
3. Best results are obtained when a constant volume is used with a set of samples and standards. Use Buffer to bring all samples to the same volume.